Asian Journal of Chemistry Vol. 17, No. 3 (2005), 1955–1958

Extraction and Characterization of *Allamanda Cathartica*

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The flowers of *Allamanda cathartica* were analyzed phytochemically and the compound was isolated from ethyl acetate extract employing chromatographic techniques. The compound was characterized by chemical and spectral methods to be flavonols and it was found to be quercetin 3-O-(6″-O-acetyl)neohesperidoside

Key Words: Extraction, Characterization, *Allamanda cathartica.*

INTRODUCTION

Allamanda cathartica belonging to Apocyanaceae is an evergreen, erect or climbing shrub. It is a native of Guyana and naturalized in many parts of central, eastern and southern India and in Andaman Islands.

EXPERIMENTAL

The plant material was collected from Tiruchirappalli district during April-May, 2004. The plant specimen was verified at Rapinat Herbarium, St. Joseph's College, Tiruchirappalli, India.

Extraction and fractionation: Fresh yellow coloured flowers (2 kg) of *A. cathartica* was extracted with 80% methanol (5 × 500 mL) under reflux. The alcoholic extract was concentrated *in vacuo* and the aqueous concentrate was successively fractionated with light petrol (60–80°C) (3 × 250 mL), peroxide-free diethyl ether (3 × 250 mL) and ethyl acetate (5 × 250 mL)[1]. The ethyl acetate fraction was taken up for study.

Ethyl acetate fraction (flavonol glycoside-quercetin 3-O-(6″-O-acetyl)-neohesperidoside).

The residue from ethyl acetate fraction was taken up in propanone and left in an ice-chest for two days when a pale yellow solid separated. It came out as pale yellow flakes (G_1) on crystallization from hot methanol, m.p. 186–87°C, yield 0.1%, and developed a greenish-brown colour with alcoholic Fe^{3+}, formed yellow precipitate with basic lead acetate solution and reduced ammonical $AgNO_3$ but

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not Fehling's solution. It developed a yellow colour when viewed under UV with and without ammonia; but did not show positive Horhammer-Hansel test[2]. It responded to Gibb's[3], Molisch's and Wilson's boric acid test[4]. It had λ_{max}^{MeOH} 257, 281, 297sh, 360; +NaOMe 273, 325, 411; +$AlCl_3$ 273, 305sh, 435; +($AlCl_3$-HCl) 269, 301, 364, 399; +NaOAc 273, 301sh, 377 and +(NaOAc-H_3BO_3) 265, 301, 372 nm. The ^1H and ^{13}C-NMR spectra of the glycoside are appended.

Acid hydrolysis of the glycoside: The glycoside (0.05 g) dissolved in hot aq. MeOH (2 mL, 50%) was hydrolysed with 5% H_2SO_4 at 100°C for about 2 h. The excess alcohol was distilled off *in vacuo* and the resulting aq. solution was extracted with diethyl ether. The residue from diethyl ether fraction was studied as presented below.

Identification of the aglycone (flavonol-Quercetin): The diethyl ether fraction was concentrated *in vacuo* and left in an ice-chest for a week. The yellow solid that separated was filtered and studied which, on recrystallization from MeOH, afforded pale yellow needles, m.p. 313–15°C (yield 0.02%). It reduced ammoniacal $AgNO_3$ in the cold and Fehling's solution on heating. It shows positive to Horhammer-Hansel, Wilson's boric acid and Gibb's tests. It gave a pentaacetate, m.p. 200–01°C and a pentabenzoate, m.p. 188–90°C. It had λ_{max}^{MeOH} 255, 269sh, 370; +NaOMe 262sh, 322, 420 (dec); +$AlCl_3$ 267, 303, 458; +($AlCl_3$-HCl) 267, 303, 351, 428; +NaOAc 275, 328, 390 and +(NaOAc-H_3BO_3) 262, 303sh, 386 nm. Its R_f values are matched with quercetin and it was confirmed by Co- and mixed paper chromatography and m.m.p. with an authentic sample quercetin.

Identification of sugar (glucose and rhamnose): The aq. solution from the above hydrolysate was neutralized with $BaCO_3$ and filtered. The concentrated filtrate on paper chromatography exhibited R_f values corresponding to those of glucose and rhamnose. The identity of the sugar was also confirmed with authentic sample of glucose and rhmanose.

RESULTS AND DISCUSSION

The UV spectrum of the aglycone obtained from the ether fraction exhibited λ^{MeOH} at 370 nm (band I) and 255 nm (band II) indicating a flavonol skeleton[5]. Its NaOMe spectrum degenerated with time. Flavonols possessing free —OH groups at 3,3′- and 4′- positions are known to be unstable in NaOMe[6]. It could therefore be inferred that there are free —OH groups C-3, C-3′ and C-4′ in the compound. A shift of 58 nm on the addition of $AlCl_3$-HCl is indicative of the presence of a free —OH at C-5 in A-ring[7].

A comparison of $AlCl_3$ and $AlCl_3$-HCl spectra revealed an additional bathochromic shift of 30 nm in the case of $AlCl_3$ spectrum (without acid), which again points to the presence of catechol type of B-ring[8]. The presence of a free —OH at C-7 is evident from the bathochromic shift if 20 nm in band II on the addition of NaOAc[9]. The presence of a catechol type of B-ring is also evident

from the bathochromic shift of 16 nm noticed in band I on addition of H_3BO_3[10, 11].

The UV spectrum of the glycoside (G1) showed two absorption maxima at 360 nm (band I) and 257 (band II). A bathochromic shift of 51 nm observed in band I of its NaOMe spectrum indicates the presence of a free —OH group at C-4′[12]. The $AlCl_3$-HCl spectrum of the glycoside four absorption maxima indicates a free —OH group at C-5[13] which is further supported by a bathochromic shift of 39 nm in its $AlCl_3$-HCl spectrum and positive response of the glycoside to Wilson's boric acid test. The presence of a free —OH group at C-7 could be inferred from a bathochromic shift of 16 nm (band II) on the addition of NaOAc. The presence of a catechol type of B-ring could be inferred from a bathochromic shift of 12 nm in band I noticed in its NaOAc-H_3BO_3 spectrum. Further, a bathochromic shift observed in the MeOH spectrum (band I) of the aglycone obtained after the hydrolysis of the glycoside as compared to that of the glycoside suggests the site of glycosylation[14] could be at C-3 which is also supported by the fact that the glycoside did not respond to the Horhammer-Hansel test whereas the aglycone did.

In the ^{1}H-NMR spectrum (500 MHz, DMSO-d_6 TMS) of the glycoside, the signal at δ 12.50 ppm can be traced to —OH at C-5. The sharp singlet at δ 10.88 ppm can be traced to —OH at C-7[15]. The signals due to C-2′ and C-6′ overlaps at δ 7.25 ppm. The C-5′ proton appears as a doublet at δ 5.60 ppm. A-ring proton at C-8 could be traced at δ 6.40 ppm. The H-1″ of glucose resonates at δ 5.20 ppm and H-1‴ of rhamnose at δ 4.55 ppm[16]. The remaining sugar protons appear in the region of δ 3.00–4.00 ppm. The appearance of methyl proton of rhamnose at δ 1.20 ppm clearly reveals the presence of neohesperidoside[17]. The signal at δ 1.90 ppm indicates the presence of 6″-O-acetyl group[18].

Supporting evidence for the structure of the flavonol glycoside is provided by the ^{13}C-NMR (500 MHz, DMSO-d_6, TMS). A comparison of the signal position of the glycoside with those of aglycone indicates that the upfield shift by 2.4 ppm clearly reveals that the glycosylation is at C-13[19]. The signal at C-6‴ of rhamnose at δ 20.00 ppm (not at 17.00 ppm) and that of C-6″ signal of glucose at δ 61.20 ppm (not at 66.80 ppm) clearly shows that the glycoside is a 3-O-neohesperidoside[20, 21]. The signals noticed at δ 170.40 and 22.0 ppm can be assigned to the carbonyl and methyl carbons respectively of an acetyl function attached to C-6″ carbon of the glucose moiety[22]. This is also confirmed by a downfield of 2.4 ppm in C-6″ resonance and an upfield shift of 3.90 ppm in the adjacent C-5″ resonance[23].

ACKNOWLEDGEMENTS

Authors are grateful to AICTE for the major research grant "Development of Indian Drugs for Inflammation and Diabetes" in order to carry out this work. One of the authors (SM) thanks Dr. S. Nilavalagan, Principal for constant support and encouragement.

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(*Received*: 23 November 2004; *Accepted:* 29 April 2005) AJC-4191